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Mitochondrial Respiration and Passive Stretch of the Diaphragm During Unilateral Phrenic Nerve Stimulation: Authors' Response

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We read with great interest the comments by Hooijman and Ottenheijm (1) regarding our article, “Effect of intermittent phrenic nerve stimulation during cardiothoracic surgery on mitochondrial respiration in the human diaphragm”.(2) The authors speculated that the stimulated hemidiaphragms stretched the inactive hemidiaphragms and influenced the dependent measure, mitochondrial respiration. The effects of direct stretch on skeletal muscle are well known and were not addressed in our article due to the 1200 word limitation for Brief Communications. We assert that passive stretch is not a confounding issue to our results for the following reasons.

To our knowledge, no one has measured the stretch imposed on an inactive human hemidiaphragm by an active hemidiaphragm, and the available animal work reports a small effect. Zahn measured indirect stretch in inactive, denervated rabbit hemidiaphragms resulting from contractions of contralateral functioning hemidiaphragms. In the supine position, the denervated hemidiaphragms' muscle length increased by only 3.3% during contraction of the contralateral muscle.(3) The authors concluded that, “the passive strain imposed by these length changes resulted in insignificant passive stress”. This degree of stretch is considerably less than many experiments using direct stretch (~ 10%) to study changes in muscle plasticity.(4) Not only was the potential stretch of the inactive hemidiaphragm small in our work, it was very brief. Our subjects underwent 1 minute of

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hemidiaphragm stimulation every hour (30 stimulations/bout) resulting in 3.4 ± 0.6 stimulation bouts and approximately 102 total contractions over 5 hours.

The authors cited their novel work in which denervated human hemidiaphragm muscle fibers maintained force generation over 8 weeks, presumably the result of indirect stretch occurring during normal breathing as evidence of a stretch effect.(5) This work did not address diaphragm mitochondrial respiration, and we did not report diaphragm fiber contractile measures, thus making comparisons between these disparate measures difficult.

The question was raised whether indirect stretch may have led to increased mitochondrial calcium and respiration in the inactive hemidiaphragms. Armstrong's work was cited in which 120 consecutive minutes of direct muscle stretch increased mitochondrial calcium and respiration in rat limb muscle.(6) In our work, the intermittently stimulated hemidiaphragms potentially stretched the inactive hemidiaphragms for ∼1.7% of the five hour experiment. Comparing the results of an *in vitro* experiment in which rat limb muscles underwent *direct, substantial* and *continuous* stretch for 120 minutes to an *in vivo* experiment that imposed *indirect, minimal* and *intermittent* stretch on human diaphragms is, in our opinion, tenuous.

In summary, we feel strongly that the minimal potential stretch, the small number of stretch cycles and the brief time that the muscles may have been affected make it unlikely that indirect stretch of the inactive hemidiaphragms confounded our conclusions. More importantly, the hypothesized effects of stretch on the inactive hemidiaphragms would have diminished the difference in mitochondrial respiration between the stimulated and inactive hemidiaphragms, and we reported a robust effect of intermittent hemidiaphragm contractions on human diaphragm mitochondrial respiration following mechanical ventilation/surgery. For these reasons, we conclude that any stretch that may have affected the inactive hemidiaphragms was likely of little or no physiological importance.

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References

1. Hooijman PE, Ottenheijm CAC. Mitochondrial Respiration and Passive Stretch of the Diaphragm During Unilateral Phrenic Nerve Stimulation. Crit Care Med. 2014 in press.
2. Martin AD, Joseph AM, Beaver TM, et al. Effect of intermittent phrenic nerve stimulation during cardiothoracic surgery on mitochondrial respiration in the human diaphragm. Crit Care Med. 2014; 42(2):e152–156. [PubMed: 24126442]
3. Zhan WZ, Farkas GA, Schroeder MA, et al. Regional adaptations of rabbit diaphragm muscle fibers to unilateral denervation. J Appl Physiol. 1995; 79(3):941–950. [PubMed: 8567538]
4. Sasai N, Agata N, Inoue-Miyazu M, et al. Involvement of PI3K/Akt/TOR pathway in stretch-induced hypertrophy of myotubes. Muscle a d Nerve. 2010; 41(1):100–106.

5. Welvaart WN, Paul MA, van Hees HW, et al. Diaphragm Muscle Fiber Function and Structure in Humans with Hemidiaphragm Paralysis. Am J Physiol Lung Cell Mol Physiol. 2011; 301(2):L228–L235. [PubMed: 21622847]

6. Armstrong RB, Duan C, Delp MD, et al. Elevations in rat soleus muscle [Ca2+] with passive stretch. J Appl Physiol (1985). 1993; 74(6):2990–2997. [PubMed: 8396114]